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THE EFFECT OF CESIUM-137 GAMMA RAYS ON REGENERATION IN *TUBULARIA*[1]

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Inhibition of regeneration by irradiation was first observed by Bardeen and Baetjer (1904). Using an x-ray tube they demonstrated that Roentgen rays had an inhibitive effect upon cell-reproduction in planarians..

Years later, Curtis and Ritter (1927) demonstrated that x-rays inhibited regeneration in *Tubularia crocea,* but a definite radiation exposure was not determined. Inhibition of regeneration by x-irradiation was also observed in the limbs of *Amblystoma* (Butler, 1931). Puckett (1936) reported complete inhibition of regeneration in colonies of *Pennaria tiarella* exposed to 10,500 R or more. Lower exposures retarded the regeneration of hydranths, but failed to check the process completely. These early experiments demonstrated that x-irradiation markedly effects regeneration.

The initial objective of this study was to determine a specific non-lethal exposure of ionizing radiation that would inhibit regeneration in *Tubularia.* Previous investigations have shown that coelenterates are highly radiation resistant, but no specific information was available to verify this fact for the marine forms.

This paper describes the response of *Tubularia* to cesium-137 irradiation. Studies were made over a wide range of exposures including lethal levels. General changes in the regeneration process were observed and characterized.

Materials and Methods

The forms of *Tubularia* used in these experiments grow in the Woods Hole, Massachusetts area throughout the summer. Only fresh material brought to the laboratory daily was used.

To induce the process of regeneration, stems 5 mm to 10 mm in length were cut from the colonies. A transverse cut was made below the hydranth and oblique cut 5 mm to 10 mm below the transverse cut. The transverse and oblique cuts were made to distinguish between distal and proximal ends of the cut stems. Cut stems were kept in glass finger bowls covered with cheese cloth and placed under running sand-filtered sea water.

Stems were irradiated using 667 Kev gamma rays from a 5000 Curie cesium-137 source at an exposure rate of 4000 R/min. All stems were irradiated in a Petri dish containing sea water and transferred to finger bowls postirradiation. Except for pilot experiments, all irradiations were completed within two hours after cutting.

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Exposures ranged from 10,000 R to 350,000 R requiring 2.5 to 87.5 minutes of irradiation.

The temperature during irradiation ranged from 10° C to 23° C. This was dependent upon the time required to accumulate a particular radiation exposure. For exposures greater than 75,000 R, iced sea water varying from 10° C to 18° C was used and changed during the irradiation procedure. Earlier observations showed that a temperature rise above 23° C during irradiation killed the organism.

Stems were observed under a dissecting microscope at different time periods post-irradiation. Seven stages of regeneration were defined. These were: pink, striated, double striated, pinched, bundle, emerging and emerged (Fig. 1). At

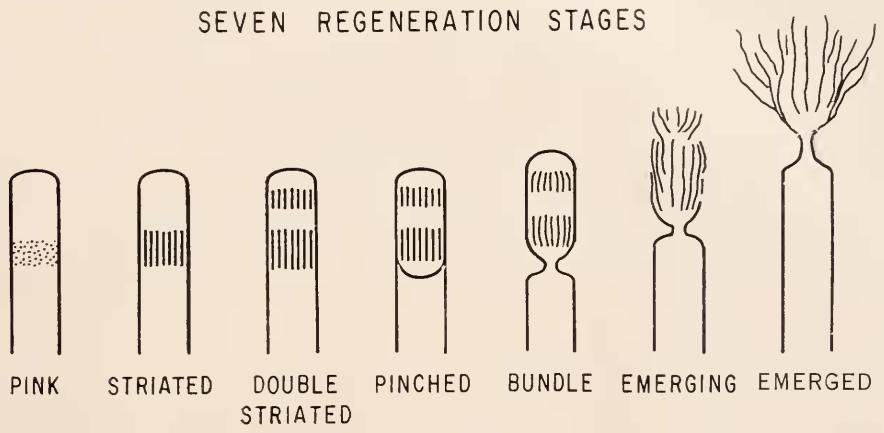

FIGURE 1. A diagrammatic representation of seven recognizable stages in *Tubularia* regeneration.

each observation time a count was made of stems in each category. Stems undergoing no regeneration were also counted. Unirradiated stems fully regenerated complete hydranths within 36 to 48 hours after cutting. All stems died when kept more than five days in this particular laboratory situation.

RESULTS

Time of irradiation

Pilot experiments were performed to establish an optimum irradiation procedure. Preliminary experiments showed that stems of *Tubularia* will undergo complete regeneration at a radiation exposure of 10,000 R, but at a slower rate when compared with unirradiated stems. Experiments were performed to determine the most effective time to irradiate the organism. Groups of 10 stems were cut within the same time interval and exposed to 10,000 R at 2, 6, 12 and 18 hours after cutting. Comparisons were made of the number of stems from each group in the emerged stage of regeneration at these times. No stems, controls or irradiated, had reached the emerged stage 24 hours aftr cutting. Forty-two hours after cutting 0, 6, 3 and 9 stems irradiated, respectively, 2, 6, 12 and 18 hours

TABLE I

Accumulated delay in regeneration at various radiation exposure levels

# of stems in each group	Exposure	Time (hours)									
		30	36	42	48	54	60	66	72	78	90
		Per cent of stems pinched and beyond at various observation times after cutting									
20	Controls	25	55	85	80	95	100	100	100	100	100
20	10,000 R	10	40	60	80	85	90	90	85	80	80
20	20,000 R	5	30	40	50	65	80	90	85	85	85
20	50,000 R	0	5	5	30	30	40	65	65	70	70
20	75,000 R	0	0	0	0	5	10	20	45	45	65
20	100,000 R	0	5	5	5	15	20	25	25	40	40

after cutting had emerged. In addition, a group of 10 stems were irradiated at 10,000 R immediately after cutting; when observed 44 hours after cutting, only one stem in this group had emerged. Based on these data, it was concluded that maximum effectiveness is achieved if stems are irradiated within two hours after cutting. The general results presented below were based on subsequent experiments in which irradiation of stems was completed within two hours after cutting.

General radiation effects

Stems irradiated at 10,000 R and 20,000 R progressed through all the stages of regeneration, but the emerged hydranths had shorter tentacles than those hydranths regenerated from un-irradiated stems. This shortening of tentacle length became more pronounced as the radiation exposure increased.

At exposures of 75,000 R and 100,000 R no tentacles developed on those hydranths that formed, even though the hypostome emerged from the perisarc. Characteristic stages of *Tubularia* regeneration are identified by striations developing at the regenerating sites. The striations are developing distal and proximal

TABLE II

Delay in regeneration at various radiation exposure levels

# of stems in each group	Exposure	Time (hours)									
		20	36	42	48	54	60	66	72	78	90
		Per cent of stems emerged at various observation times after cutting									
20	Controls	0	1	35	50	80	90	100	100	100	100
20	10,000 R	0	0	5	35	55	90	90*	90	90	90
20	20,000 R	0	0	0	15	35	65	70	90*	90	90
20	50,000 R	0	0	0	0	20	25	50	40	65	70
20	75,000 R	0	0	0	0	0	5	15	25	40	65
20	100,000 R	0	0	0	0	5	10	20	25	35	40

* Indicates that at later observations emerged hydranths had begun to drop off.

tentacles. The lack of tentacle growth at high exposures made the various stages of regeneration less recognizable.

Observations and comparisons of regenerated hydranths showed that hydranths regenerated from irradiated stems tended to drop off sooner than those hydranths regenerated from un-irradiated stems.

Stems were exposed to 50,000; 75,000; 100,000; 150,000 and 200,000 R to determine an exposure that completely inhibits regeneration. Observations of the stems after irradiation indicated that 150,000 R and 200,000 R inhibit regeneration, but were not lethal to the organism 90 hours after cutting.

The inhibitory effect of irradiation appeared to be dose dependent. As the exposure increased, the delay in the time of regeneration increased. From recordings of the number of stems in each stage of regeneration for each exposure, all stages appeared to be uniformly affected by irradiation. Table I is a summary of data on delay in regeneration at various radiation exposure levels based on the percentage of stems completing the pinched and all subsequent regeneration stages within the given observation intervals. Table II summarizes data on the observed delay in regeneration time of emerged stems at various exposure levels. In this experiment those irradiated stems that did emerge were, respectively, 6, 12, 18 and 30 hours behind the un-irradiated stems. Figure 2 is a graphic representation of the data in Table II. The percentage of stems completing the emerged stage of regeneration decreases as the exposure levels increase.

Stems were exposed to radiation levels ranging from 225,000 R to 350,000 R in steps of 25,000 R. At an exposure of 300,000 R, 10 out of 20 stems showed

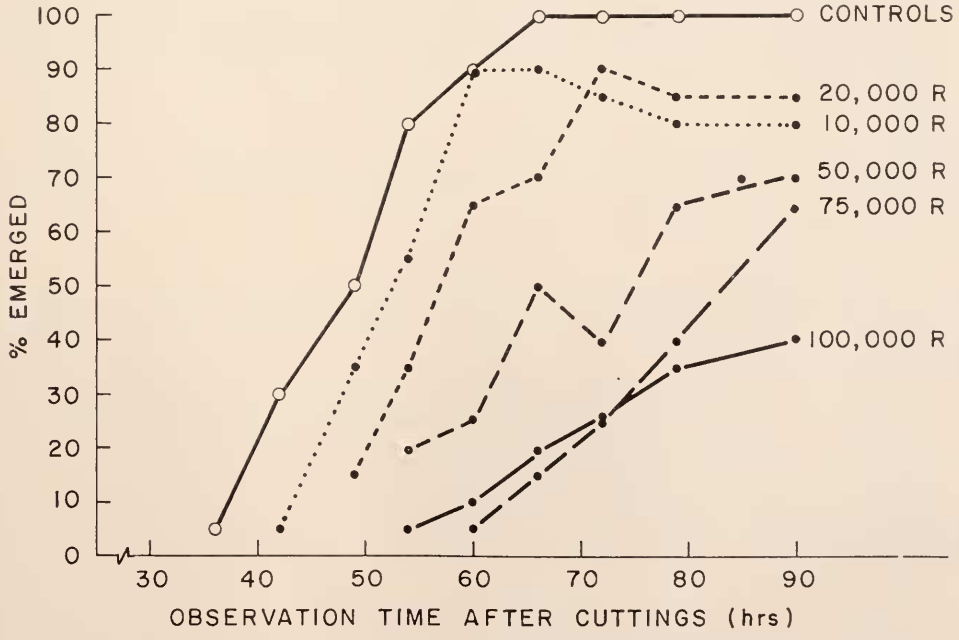

FIGURE 2. A comparison of the percentage of irradiated and control stems in the emerged stage of regeneration at different observation times after cutting.

signs of tissue disintegration when observed six hours after cutting. At eighteen hours after cutting and irradiating all 20 stems were dead. Exposures of 300,000 R and above are fatal to the organism under the given experimental conditions. For the various experiments described a total of 900 organisms was used in ten distinct experiments.

Discussion

It is well-known that ionizing radiation affects regeneration in various invertebrates and vertebrates. Irradiation has frequently been used as a means of studying biological mechanisms involved in the regeneration process. The quantity of radiation necessary to inhibit regeneration is extremely variable. In the coelenterates, various genera react differently; some coelenterates are more radiation resistant that others. X-irradiated hydra whose interstitial cells have been destroyed are still capable of limited regeneration. (Brien and Reniers-Docoen, 1955). Also for the hydra, an exposure of 4500 R inhibits bud formation (Park, 1958). Colonies of *Pennaria tiarella* require 10,500 R to inhibit regeneration. For the colonial hydroid, *Tubularia*, an exposure of 150,000 R is required to inhibit regeneration.

Inhibitory exposures have also been determined in various other organisms. Regeneration in the polychaete, *Clymenella torquata* is inhibited at an exposure of 50,000 R (Rose, F., unpublished observations). X-irradiation ranging from 1000 R to 10,000 R when applied locally will prevent limb and tail regeneration in the adult urodele, *Triturus* (reviewed by Rose, 1964).

The lethal exposure of 300,000 R observed in the present study for *Tubularia* is in the range of exposures required to deactivate many viruses. Viruses lose their infectivity when exposed to radiations ranging from 430,000 R to 5,150,000 R (Luria, 1951).

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Summary

1. Short tentacles developed on hydranths regenerated from stems of *Tubularia* exposed to cesium-137 gamma irradiation at exposure levels of 10,000 R and 20,000 R.

2. Those hydranths that did regenerate from irradiated stems tended to drop off sooner than hydranths regenerated from un-irradiated stems.

3. Exposures of 150,000 R and 200,000 R completely stop regeneration in *Tubularia*; 300,000 R is fatal to the organism.

4. The observed delay in time of regeneration increased as the exposure levels increased and thus appeared to be dose dependent.

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